GLASS CERAMIC MATERIALS FOR MEDICINE

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GLASS CERAMICS WITH CONTROLLABLE PORE STRUCTURE FOR MEDICINE

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The conditions for synthesis of porous biocomposite materials based on calcium phosphate for fabricating implants with prescribed pore-structure parameters, and the desired level of physical, chemical, and biological properties for clinical applications are determined. The role of a bimodal pore structure in the substrate materials for cell cultures is shown.

Modern medical materials-science technologies are continually developing, and with each passing year newer and more promising materials suitable for application in reconstructive medicine are being developed.

Materials which are used as carriers or substrates for cell and bacteria cultures have started to appear in the last few years. Such materials are used in various fields ranging from medicine to the defense industry and for ecological purposes.

A consequence of the development and advancement of cell biology was the organization of a new direction of cellular and tissue engineering, concerning biomedical technology, based on the use of cultured human cells. The problems of this direction are to provide whatever is necessary to replace and restore damaged tissues by implantation or transplantation of grown (*in vitro*) cells from healthy organs and tissues. The fact that cells can be cultured in quantities adequate for practical purposes makes it realistic to use them in clinical medicine.

A cell culture is a method of long-term maintenance and growth of various types of cells in special nutrient media [1]. Cell cultures are maintained for indefinite periods of time by continual transference, and they have specific properties and indicators which are maintained during culturing. Cellular material induces and intensifies the regenerative potential maintained in the patient's own cellular systems, and it materializes itself not only in replacement therapy but also in organizing-inducing therapy.

Polymers, metals, glass, and other materials with different chemical composition and of different nature are used as carriers of cell cultures. Experience has shown that the chemical composition of the material plays an important role in creating the preferred conditions for cells to live. The principal factors that have a positive effect on cell maintenance and division is the texture and pore structure of the substrate material [2, 3]. The most promising materials for substrates and backings for culturing different types of cells are porous polymer and inorganic materials.

The goal of the present work is to develop a composition and technology for fabricating a porous biocomposite material (BCM) which can be used as a carrier substrate for human cell cultures in order to develop implants for replacing defects in bone tissue. Such so-called "live implants" are intended to accelerate the process of osteogenesis, increase the bioactivity of the material, and decrease the patient's treatment time.

The medical immunobiological preparation "Cultures of human diploid cells for replacement therapy," which is a strain of human diploid cells that was obtained in the Ekaterinburg Scientific–Research Institute of Viral Infections (ENIIVI) of the Ministry of Health of the Russian Federation by a group of authors headed by N. P. Glinskikh (USSR Inventor's Certificate No. 1147748). There is a complete data sheet for this culture, according to the requirements of the International Association of Cell Cultures, and the culture has been registered as an original and maintained strain LÉCh 4(81) at the low-temperature bank-museum ENIIVI. The preparation is a morphologically uniform population of cells with a limited life span, definite tissue originating partial differentiation, and a fibroblastic phenotype. For safety, the preparation is checked for viruses and bacteria, and its sterility is monitored following MUK 4.1/4.2

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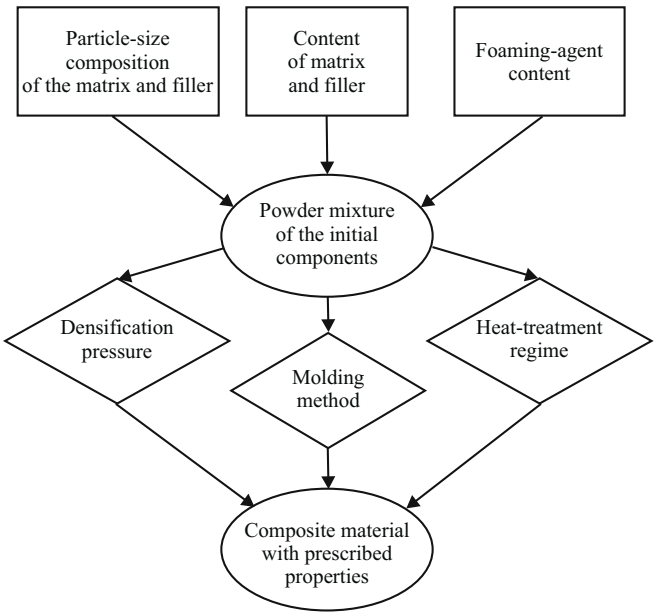
588–96. An assessment of any propensity for carcinogenicity and tumor-induction was made according to WHO and SP 3.3.2.561–96 requirements.

The calcium phosphate bioactive porous composite material developed as a substrate-backing for cell cultures consists of a neutral silicate matrix containing the following (%[2]): 73.0 SiO_2, 3.5 Al_2O_3, 2.5 B_2O_3, 1.0 MgO, 7.0 CaO, 11.0 Na_2O, 2.0 L_2O, a filler consisting of granular hydroxyapatite (HAp) with Ca : P = 1.67, and a carbonate foaming agent.

Studies of the initial components for obtaining a composite material showed that the average interval of the sintered state of the matrix glass is 250°C, and sintering of the glass powder without plastic deformation of the samples occurs at temperatures from 700 to 780°C. When a foaming agent is added in amounts 3 – 4% foaming of the glass occurs in the temperature range 760 – 850°C with uniform porous cellular structures with a predominantly open porosity being formed. The temperature interval of glass sintering and foaming corresponds to the range of thermal stability of the bioactive phase of the composite material formed — HAp.

The principal properties of the BCM developed — a bimodal pore structure and porosity, which depend on the ratio of the components of the materials (matrix : filler) and the particle-size distribution — vary over ranges (see diagram) [4].

A strong bond is formed between the glass matrix and the filler during sintering when an amount of glass melt sufficient for liquid sintering is present. This quantity is determined by a sinterability index, which is introduced in the present work, S_t defined as the ratio of the specific surface areas of the sintered fractions of the glass matrix and filler. It was determined experimentally that there is a range of optimal values of S_t for each particle-size composition of the filler and increases from 5 to 135 as the sizes of the filler granules increase from 10 to 900 μm. Figure 1 shows S_t as a function of the size of the filler granules and the content of the filler in the material. As the size of the HAp granules increases, the range of working values of S_t expands as a geometric progression.



Technological conditions for obtaining a composite material with prescribed properties.

Studies of the BCM obtained show that as the matrix content decreases and the HAp grain-size increases from 10 – 50 μm the bulk mass of the material decreases because the total porosity increases. At the same time the rate of permeation of a physiological solution into the material increases substantially — from 3 to 25 mm/min. The open and closed porosities of the experimental samples depend on the ratio of the glass matrix and the filler within the experimental range of the filler particle-sizes. When fine filer fractions are

[2] Mass content, here and below.

TABLE 1.

Sample*	HAp fraction, μm	Matrix : filler ratio, %	S_t	Volume mass, g/cm^3	Water absorption, %	Porosity, %			Permeation rate, mm/min
						total	open	closed	
1.1	10 – 50	80 : 20	7	2.06	4	24	7	17	0.13
1.2		70 : 30	4	1.61	21	41	34	7	3.33
1.3		60 : 40	3	0.33	54	76	35	41	0.34
2.2	50 – 200	70 : 30	14	1.21	25	54	30	24	11.30
2.3		65 : 35	11	1.21	33	55	40	15	9.80
2.4		60 : 40	9	1.30	29	52	38	14	25.00
3.1	200 – 600	80 : 20	98	0.47	40	73	28	44	0.30
3.2		70 : 30	57	0.64	36	71	27	44	0.54
3.3		60 : 40	38	0.98	35	65	33	32	2.50
3.4		50 : 50	25	0.88	54	67	50	17	2.50
4.1	600 – 900	60 : 40	66	0.61	35	40	33	16	3.00

* The average values with confidence interval ± Δ 0.5% are presented.

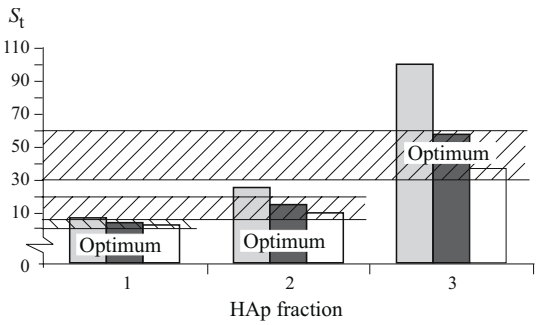


Fig. 1. Sinterability index S_t versus the particle-size fraction of the filler in the initial powders with different ratio of the matrix and the filler: *1*, *2*, *3*) HAp fractions 10 – 50, 50 – 200, and 200 – 600 μm, respectively; Optimum) zones of working compositions; □, ■, □) ratio of the matrix and filler 80 : 20, 70 : 30, and 60 : 40%, respectively.

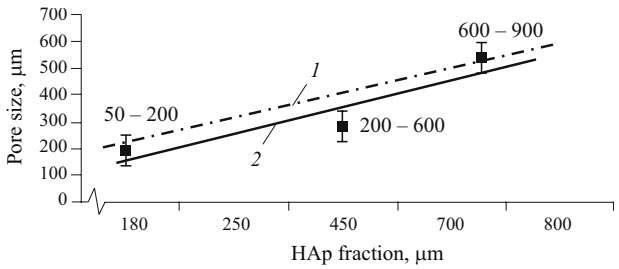


Fig. 2. Pore size versus the initial filler particle sizes with the ratio matrix : filler = 60 : 40%. The curves were constructed using the theoretical (*1*) and experimental (*2*) data.

used, closed porosity predominates; this decreases the permeability and resorbability of the material. The closed-pore fraction decreases within the range of large filler particle sizes and reaches 20 – 40% on average with total porosity up to 60 – 65%. This shows that the permeability and biological activity of the material are secured by fillers with grain sizes 200 – 600 μm and an adequate closed-pore fraction (up to 30%).

The porosity with glass mass content 70 – 80% is essentially closed, as a result of which the permeability of the material is very low. This shows that crystalline HAp granules form a framework when the filler content is at least 40%. In this case, the open-pore fraction increases and, correspondingly, the permeation rate increases.

Examinations of the structure of the samples in a microscope showed that the pore structure depends on the filler content in the range from 20 to 40% and the filler granule size. The experimental dependence of the pore size on the filler grain size agrees with the computed relation (Fig. 2).

Thus, the porous BCM developed with filler granules 200 μm or more in size possesses a bimodal cellular–porous structure. Two types of pores are observed.

One type consists of cells which appear in the material as a result of the intergrain voids of the filler granules. The cell size is determined by the granulometric composition of the filler. The formation of these pores is controlled by selecting a prescribed filler particle-size fraction and can be controlled within the range of the compositions studied with filler-grain size 100 – 900 μm. As the size of the filler granules increases from 200 to 900 μm and the content of the granules in the initial mixture increases, the pores increase in size and number. As the granule size decreases from 200 to 50 μm, pores form in the material as a result of foaming of the glass matrix. In this case, the pore size lies in the range 100 – 500 μm, which is sufficient for bone cells to colonize the pores.

The other type consists of pores smaller than 100 μm. Such pores are formed when the decomposition of the carbonate foaming agent makes the glass matrix foamy and as a result of the intrinsic microporosity of the filler granules, which makes it possible to control the formation of open pores of this type and increase the permeability and resorbability of the BCM.

The porosity and its character can also be controlled in structures like live bone. For this reason, a fragment of the transverse cross section of a vertebra with a unidirectional porosity gradient ranging from a dense microporous cortical layer to a spongy layer with pore size ranging from 50 to 500 μm was chosen as a model of the BCM being developed with a size-differentiated regulatable pore structure (ORION-MB) oriented according to the character of the pore distribution. Some examples of the ORION-MB compositions for different types of implants and their characteristics are presented in Table 2.

TABLE 2.

Implant	Water absorption, %	Cortical layer (C)			Spongy layer (S1)			Spongy layer (S2)		
		sample*	filler fraction, μm	matrix : filler ratio, %	sample*	filler fraction, μm	matrix : filler ratio, %	sample*	filler fraction, μm	matrix : filler ratio, %
É1	35	2.4	50 – 200	60 : 40	3.3	200 – 600	60 : 40	–	–	–
É2	35	3.3	200 – 600	60 : 40	3.2	200 – 600	70 : 30	–	–	–
É3	36	1.2	10 – 50	70 : 30	3.3	200 – 600	60 : 40	3.2	200 – 600	70 : 30
Ts1	35	3.3	200 – 600	60 : 40	3.2.2	200 – 600	70 : 30	–	–	–
Ts2	32	1.2	10 – 50	70 : 30	3.2	200 – 600	70 : 30	–	–	–

* The foaming-agent content per 100 g is 4% in the mixture and 2% in sample 3.2.2.

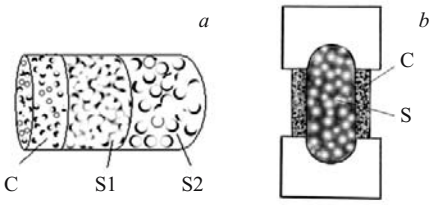


Fig. 3. ORION-MB multilayered materials: *a*) elliptic implant or type-É BCM; C) cortical or moderately porous layer (porosity 30 – 50%); S1, S2) spongy or highly porous layer (porosity 50 – 70%); *b*) cylindrical implant or type-Ts BCM; C) outer cortical layer or moderately porous layer (porosity 30 – 50%); S) inner spongy or highly porous layer (porosity 50 – 70%).

A multilayered BCM was obtained by combining layers with different granulometric composition using different filling methods, as a result of which different types of samples were made (Fig. 3).

Our investigations showed that fractions with close sinterability index, thermal shrinkage, and foaming should be used to obtain multilayered porous samples. When sintering layers with different porosity it is best to lower the content of the foaming agent in compositions of the inner layers and thereby weaken the strength of the foaming agent in the inner foaming layer, which, in turn, will decrease the risk of the sample fracturing along the outer or inner layer and undesirable cracks appearing.

Experiments performed in three series of multilayered samples established that for sintering with the participation of a liquid phase all patterns and mechanisms of the formation of porous structures of various types operate in the multilayered system obtained.

Biological investigations of samples were performed to determine the possibility of implanting medical immunobiological preparation "Human diploid cell cultures for replacement therapy" on them.

A diploid cell culture in a stable stage of development in an active growth phase was used for the analysis. The culture was transferred to the material (transference) assuming 100,000 cells per 1 cm^2 of the sample. The cell concentration used in the experiment ensured that a monolayer would form in the control experiment in 3 – 5 days of growth. The material was soaked for 24 h in the maintenance medium used for the cell culture to stabilize the chemical composition and normalize the pH. Next, the maintenance medium was poured off and the material was transferred into a culturing flask with a suspension of cells in the growth medium. The rate of increase of the number of cells was determined from the change of the pH of the growth medium as compared with the data from the control experiment (growth of cells on a neutral glass) by the method "Separation, culturing, and monitoring strains of diploid cells," developed by the Ministry of Health of the Russian Federation.

Since the structure of the material did not permit a direct morphological analysis to be performed on the material itself, the material was removed from the culture flask, rinsed in Hank's solution to remove unattached cells, and transferred into a clean culture flask with a growth medium and a small piece of cover glass. The cells, which continued to grow, spread from the material onto the glass and formed a monolayer, whose rate of formation is an additional criterion for cell activity. Next, the glass was removed for morphological analysis. The morphological indicators were determined using generally accepted procedures. The suitability of different substrates for growing cell cultures is reflected in Table 3.

TABLE 3.

Sample	Open porosity, %	Permeation rate, mm/min	Cell growth
1.2	83	3.3	Active
1.3	46	0.3	No
2.4	73	25.0	Active
3.2	38	0.5	Weak
3.3	51	2.5	Active

Our investigations have shown that the active growth phase of a culture of human diploid cells proceeds best with high open porosity and permeability.

In summary, the bimodal porous structure of the material plays an important role in creating the preferred conditions for maintaining and growing cells. For example, the sample 1.3, which contained predominately large pores (> 500 μm), gave a negative result while active cell growth occurred in all other samples where, aside from 100 – 500 μm pores, a quite large number of pores smaller than 100 μm were present.

Biological tests performed on the one-layer and multilayer porous biocomposite materials developed have shown these materials to be suitable for carriers of cell cultures and as a base for fabricating "live implants," positively affecting the degree of their osteointegration in the body. The materials developed make it possible to implant in a patient a material with cell cultures which have already been seeded and taken hold and which increase the bioactivity of the material and accelerate the colonization of the material with bone cells.

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